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A novel *SCN1A* mutation associated with severe GEFS+ in a large South American pedigree

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Summary Generalized epilepsy with febrile seizures plus (GEFS+) is an inherited epileptic syndrome with a marked clinical and genetic heterogeneity. Here we report the molecular characterization of a large pedigree with a severe clinical form of GEFS+. Genetic linkage analysis implied the involvement of the FEB3 in the disease phenotype of this family (parametric two-point lod-score of 2.2). Sequencing of the *SCN1A* gene revealed a novel aspartic acid for glycine substitution at position 1742 of this sodium channel subunit. The amino-acid replacement lies in the pore-forming region of domain IV of SCN1A. Our observations are consistent with the genotype–phenotype correlation studies suggesting that mutations in the pore-forming loop of SCN1A can lead to a clinically more severe epileptic syndrome.

Introduction

Generalized seizures during febrile episodes (febrile seizures, FS) occur in 2–5% of children under six.[1] Of these, about 5% develop non-febrile seizures and/or epilepsy later in life (denoted as FS+).[2] Family studies indicate an important genetic susceptibility to FS[3] and have led to the identification of four linked loci: FEB1 at 8q13-q21,[4] FEB2 at 19p13.3,[5] FEB3 at 2q23-q24,[6] and FEB4 at 5q14-q15.[7] Scheffer and Berkovic[8] defined an autosomal dominant form of FS+ denoted generalized epilepsy with febrile seizures plus (GEFS+) to include individuals with various types of seizures (partial, absences, astatic). Mutations at four genes resulting in GEFS+ have recently been identified: the type-1 voltage-gated sodium-channel subunits β (*SCN1B*) and α (*SCN1A*),[9,10] the gamma-2 subunit of the gamma-aminobutyric acid receptor (*GABRG2*)[11] and the type-2 voltage-gated sodium-channel subunit α (*SCN2A*).[12] Mutations at these genes account for less than 10% of families with GEFS+, indicating a further genetic heterogeneity of this epileptic syndrome.

Here we report the genetic characterization of a large South American family including several individuals with a severe form of GEFS+. Linkage analysis to known FEB genes/regions was consistent

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with linkage to the FEB3 locus. Sequencing of the exons of the *SCN1A* gene revealed a c.5213A>G mutation leading of the substitution of an aspartic acid for a glycine at position 1742 of the SCN1A protein. This mutation is located in the highly conserved pore region of domain 4 of this sodium channel subunit. Previous studies have shown that de novo mutations in this domain of the *SCN1A* protein are often associated with severe myoclonic epilepsy of infancy (SMEI), an intractable form of epilepsy that has been proposed as part of the GEFS+ spectrum.[13]

Patients and methods

Study pedigree

The index case of the family (Fig. 1) is a patient attending the pediatric neurology service of Hospital Universitario San Vicente de Paul (Medellin, Colombia). The pedigree was extended focusing on a history of seizures in relatives of the index case. All available individuals with a positive history underwent a full neurological examination and the characteristics of the seizures evaluated by interviewing relatives and reviewing available clinical records. Seizures were categorized according to international classifications.[14] Blood samples were collected from all available individuals and genomic DNA obtained using the QIAGEN DNA extraction kit following the manufacturer's instructions. The Bioethics Committee of Universidad de Antioquia approved this research project and all participants provided their informed consent.

Genotyping and linkage analysis

The following six microsatellite markers were genotyped (as described) in order to assess linkage to known FEB loci and GEFS+ genes: D8S530 for FEB1,[4] D19S177 for FEB2,[5,15] D2S382 for FEB3/*SCN1A*,[16] D5S644 for FEB4,[7] D19S425 for SCN1B,[9] and D5S1403 for *GABRG2*.[11] FEB3/*SCN1A* was followed-up by genotyping two additional markers (D2S2330 and D2S2157).[16,17] PCR reactions were performed in an MJ Research (DYAD™) thermocycler in a final volume of 15 μL with 30 ng of DNA, 0.3 μM of each primer and 1× of HotStart Taq™ Master Mix (QIAGEN). Amplification products were examined on an ABI-377 DNA sequencer (Applied Biosystems) and runs analyzed with GENESCAN and GENOTYPER software (Applied Biosystems). Two-point lod-scores were calculated using the MLINK program[18] assuming an autosomal dominant mode of inheritance with 96% penetrance (assumed from the pedigree), a mutated allele frequency of 0.0001 and equal allele frequencies at the marker loci. All individuals with a diagnosis of FS or GEFS+ were considered as affected in the analyses and a phenocopy rate of 3% was assumed.[19]

Mutation screening

All exons of the *SCN1A* gene were amplified using the primers and PCR conditions reported by Malacarne et al.[20] PCR products were sequenced using the BigDye Deoxy Terminator Cycle sequencing kit (Applied Biosystems) on an ABI-377 DNA sequencer following the manufacturer's instructions. Mutation screening was carried out by restriction enzyme digestion of PCR products with *Tsp45I* (New England Biolabs) at

Table 1 Clinical features of the patients in the family studied.

Individual	Age	FS onset/offset	FS episodes	AFS onset/offset	AFS episodes	Seizure type	Mental retardation
II 1	53	6m/7y	###	–	–	GTCS	No
II 6	59y	6m/9y	N.I.	33y/54y	N.I.	PS	No
III 3	36y	3m/N.I.	~15	N.I./ongoing	~20	GTCS	No
III 4	19y	6m/7y	###	7y/19	>20	GTCS	Yes
III 5	27y	6m/8y	###	13y/21y	###	GTCS	Yes
III 10	30y	2y/ongoing	###	30y/ongoing	###	PS	Yes
III 13	23y	9m/2y	~15	2y/ongoing	###	GTCS	Yes
III 14	7m	4m/7m	4	–	–	GTCS	No
III 16	28y	6m/ongoing	~20	8y/ongoing	###	PS	No
IV 1	11y	5m/ongoing	~20	–	–	GTCS	No
IV 3	4y	9m/3y	~10	–	–	GTCS, myoclonic	No
IV 6	10y	6m/3y	>20	–	–	GTCS	No
IV 7	3y	3m/ongoing	###	3y/ongoing	###	Myoclonic, astatic	Yes

FS: febrile seizures, AFS: afebrile seizures, GTCS: generalized tonic-clonic seizure, PS: partial seizure, y/m: years/months, ###: countless, N.I.: no information. Individuals shown in bold are deceased.

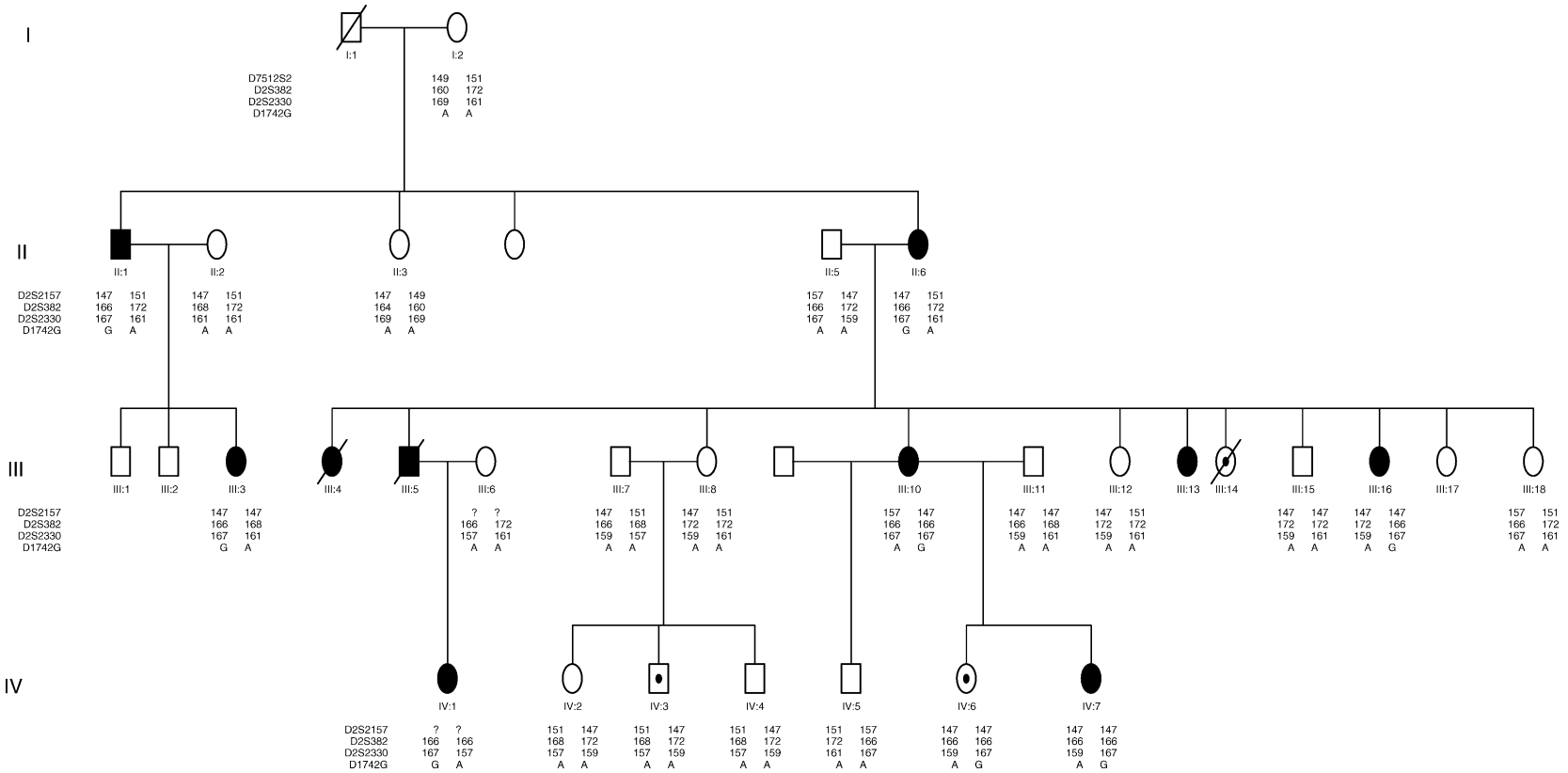

Figure 1 Pedigree of the family studied. Filled symbols indicate individuals with FS+, symbols with dots indicate individuals with FS and clear symbols represent unaffected individuals. Squares: males, circles: females. Numbers under the symbols refer to allele sizes at the loci examined. The nucleotide change leading to the D1742G substitution in SCN1A is shown as G (mutant) or A (wild-type).

Table 2 Two-point lod-scores for markers at FS loci (FEB) and GEFS+ genes.

Marker	Locus/gene	Recombination fraction				
		0	0.1	0.2	0.3	0.4
D8S530	FEB 1	−4.09	−1.53	−0.64	−0.25	−0.08
D19S177	FEB 2	−2.01	−0.68	−0.34	−0.20	−0.11
D2S382	**FEB 3/SCN1A**	**2.24**	1.83	1.39	0.92	0.44
D5S644	FEB 4	−2.19	0.04	0.42	0.47	0.32
D19S425	SCN1B	−3.62	−1.51	−0.76	−0.34	−0.11
D5S1403	GABR G2	−6.70	−2.59	−1.27	−0.57	−0.18

37 °C overnight. Digestion products were then examined on 3% agarose gels.

Results

Clinical findings

The index case is a patient aged 3 that since three months of age presents myoclonic astatic seizures (up to four per week) that have not responded to treatment with valproic acid and phenobarbital. The patient presents mental retardation and has a normal interictal EEG and CT scan. Pedigree extension revealed a history of seizures in 12 relatives of the index case, 9 of which are alive (Fig. 1). The main clinical features of the affected individuals in this pedigree are summarized in Table 1. All cases had a history of febrile seizures with the first episode occurring between 3 months and 2 years. The nine patients currently over six years of age have presented episodes of febrile seizures after that age. Eight of these individuals also presented non-febrile seizures, usually starting after age six. The most common seizures type is generalized tonico-clonic, although three patients presented complex partial seizures. Response to treatment was usually poor and most patients had a history of countless seizures, with the most recent episode ranging between 19 and 54 years of age. Mental retardation was present in four cases. Available interictal EEGs are normal.

Linkage analysis

Genotype information was obtained for 20 individuals including the nine living affecteds. Significantly negative lod-scores (<−2) were observed at theta 0 for all markers tested with the exception of D2S382 at FEB3/*SCNA1*, which showed a maximum lod score of 2.24 (Table 2). Genotypes at two additional markers in FEB 3 (D2S2330 and D2S2157) resulted in maximum lod-scores (at 0 recombination fraction) of 2.83 and 1.19, respectively. All affected individuals except IV 3 carry a haplotype characterized by alleles 147, 166 and 167 at markers D2S2157, D2S382 and D2S2330, respectively (Fig. 1). A recombination event between markers D2S2157 and D2S382 was observed in individual III 15 who is unaffected but carries the disease-associated allele 147 at locus D2S157.

Mutation identification

Sequencing of *SCN1A* exons in two carriers of the disease-associated haplotype (II 6 and III 10) revealed a heterozygous A>G substitution in exon 26 at position 5213 of the *SCNA1* mRNA (c.5213A>G, Fig. 2). This base change destroys a recognition site for enzyme *Tsp45I*. Restriction enzyme digestion of exon 26 PCR products showed that the c.5213A>G base change is present in the individuals that were considered affected, except for IV 3, which therefore represents a FS phenocopy. This DNA mutation

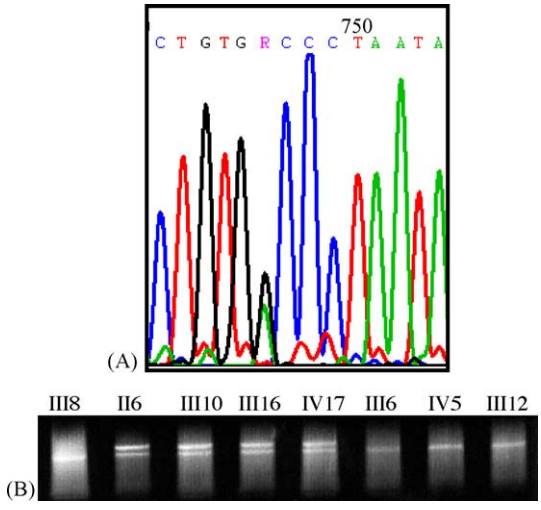

Figure 2 Mutation screening of exon 26 of SCN1A. (A) Partial nucleotide sequence of the exon showing the heterozygous A>G substitution observed in a patient sample (indicated by the letter R). (B) Restriction digestion of exon 26 with Tsp45I. The presence of the c.5213A>G mutation leads to the destruction of the restriction site. Labels of lanes refer to the individuals examined as in the pedigree of Fig. 1.

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SCN1A_HUMAN    TFGNSMICLFQITTSAGWDGLLAPILNSKPPDCDPNKVNPGSSVKGDCGNPSVGIFFFVS
SCN2A_HUMAN    ..........................G......D.DH.....................
SCN3A_HUMAN    ..........................A......DTIH.....................
SCN4A_HUMAN    .....I....E...........N.....G.......LE...T..........I..C..C.
SCN5A_HUMAN    ..A...L..............S....TG..Y...TLP.S-NGSR....S.A...L..TT
SCN9A_HUMAN    ..................................K..H.....E...........Y...
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Figure 3 Aminoacid sequence alignment of the loop between segments S5 and S6 in domain D-IV of human neuronal sodium channels. The aspartate residue (D) at position 1742 that is substituted by glutamate in the GEFS+ family examined is underlined in bold.

was not observed in 60 unaffected controls from the same population. The c.5213A>G substitution leads to the replacement of an aspartate for a glycine at position 1742 of SCNA1 (D1742G). This amino-acid lies in the evolutionarily conserved pore-forming loop between segments S5 and S6 of domain IV in the SCNA1 channel (Fig. 3).

Discussion

In the past few years, four genes have been identified that when mutated lead to the development of autosomal dominant GEFS+: *SCN1B* (GEFS+1), *SCN1A* and *SCN2A* (GEFS+2), and *GABRG2* (GEFS+3). Thus far, nine amino-acid substitutions leading to GEFS+2 have been identified in *SCNA1*.[10,13,21,22] Three of these mutations have been shown to result in hyperexitability of the sodium channel,[23] indicating that the dominance of these alleles is related to a gain of function. Recently, mutations in *SCNA1* have also been shown to underlie SMEI.[24,25] Consistent with these molecular findings, it had been suggested that SMEI could constitute the most severe form of the GEFS+ clinical spectrum.[13] The initial SMEI-causing mutations identified in *SCNA1* were nonsense or splice-site substitutions leading to the production of a truncated sodium channel and suggesting that the severity of the phenotype related to haploinsufficiency.[24] More recently, a number of *SCNA1* missense mutations have been identified in SMEI patients.[25–28] Interestingly, most of these amino-acid substitutions are located in the functionally critical pore region of SCNA1 (S4–S6) and could therefore have a major impact on the kinetic properties of this sodium channel.[25]

The molecular genetic characterization of the South American family examined here evidenced linkage to *SCN1A* and led to the identification of a novel D1742G mutation in the loop between segments S5 and S6 in domain IV of this sodium channel. The findings in this pedigree are consistent with the view that aminoacid substitutions in the pore region of SCNA1 can lead to a severe epileptic phenotype. This family includes several patients with marked clinical manifestations. The index case has astatic epilepsy, while other family members present frequent seizure episodes that have continued into adulthood and that show poor response to treatment. Several individuals also have mental retardation, although this could relate to the high frequency of epileptic episodes. Interestingly, although most affected family members have severe clinical manifestations, some individuals carrying the D1742G mutation show a milder phenotype (Fig. 1; Table 1). Most notably, the sib of the severely affected index case, presents only mild clinical manifestations (i.e. FS). This suggests that genetic and/or environmental modifiers can have an important impact on the phenotypic expression of the D1742G mutation. Identification of these modifying factors will require the study of a large number of GEFS+ patients that are carriers of this mutation. In conclusion, our findings are consistent with proposed genotype–phenotype correlations based on analyses of GEFS+ and SMEI patients but also underline the highly variable phenotypic expression of SCN1A mutations.

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